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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/532,998	11/10/2005	Konstantin Konstantinov	07430-00150-USU	1655

23416 7590 06/10/2009

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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1656	

MAIL DATE	DELIVERY MODE
06/10/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/532,998	KONSTANTINOV ET AL.	
	Examiner	Art Unit	
	SUZANNE M. NOAKES	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 March 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of the Claims

1. The amendments filed 06 March 2009 are acknowledged. Applicants have added new claim 12, thus, claims 1-12 are pending and subject to examination.

Withdrawal of Rejections/Objections

2. Any rejection/objection recited in the previous Office action and not explicitly recited below is withdrawn.

Maintained - Modified Rejections – Modification Necessitated by Amendments

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1-11 and new claim 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moller et al. (US 6103502 – cited on IDS of April 2008) in view of Schulz et al., 1997 (cited in the IDS filed 28 April 2005) and Palomares et al. (Enzymes and Microbial Technology, 2000 Mar 1;26(5-6):324-331 – cited previously) and evidenced by Nemeth et al. (Colloids and Surfaces A: Physiochemical and Engineering Aspects, 1997, 127: 151-162).

The maintained rejection is recited in the previous Office action (see Section 8) but is reiterated/mofied below for convenience.

Moller et al. teach that the need to isolate a protein or peptide from fermentation medium typically arises in the context of recombinant microorganisms transformed with suitable expression vectors. Desired proteins or peptides, for the taught ultrafiltration process, are typically a recombinantly produced protein or peptide (see col. 1, lines 19-24). The method of concentrating a macromolecule of interest via ultrafiltration of a cell culture supernatant encompasses subjecting said cell culture supernatant (from a decanted culture medium – see col. 5, lines 4-7) wherein said first supernatant (e.g. retentate) is adjusted to have a conductivity of less than 6 mS/cm and is subjected to a first ultrafiltration process which is immediately followed by diafiltration (e.g. desalinization by addition of water) until the conductivity is adjusted to less than 2.2 mS/cm. This second retentate can then be subjected to a second concentration/ultrafiltration step (see col. 5, lines 7-48).

Moller et al., however, do not teach adding an organic nonionic block copolymer such as Pluronic F-68 to the cell culture mediums such as animal cell cultures or insect cell cultures.

Schulz et al. teach that ultrafiltration (UF) is one of the most efficient processes recommended as a first step in downstream procedures for the recovery of proteins from mammalian cell cultivation. Cell culture supernatants consist of a broad spectrum of compounds, which influence the performance of UF. These include supplements of the culture medium (e.g. Pluronic F-68, silicone oil) or compounds that are secreted

from the cells or released after cell lysis (e.g. proteins, lipids). The nonionic block copolymer Pluronic F-68 is often supplemented in cell culture media for animal cells in order to protect the cells from shear stress caused by sparging. Figure 1 discloses the overall method wherein cell culture (either CHO cells +/- F-68 and SM1F6 cells +/- F-68 wherein the protein of interest is gp220/350 Epstiein-Barr-Virus surface antigen) are processed through ultrafiltration and/or subsequently through diafiltration (see Figures 1 and also 5).

Palomares et al. teach the following (see p. 324, 1st column, 1st paragraph):

> "Pluronic F-68 (PF68) has been widely used as a shear protective agent for animal cells in suspension culture. Its use has resulted in increased cell survival and cell concentration, particularly in cultures with serum-free media or in bioreactors where cells are subjected to deleterious shear stresses. The effectiveness of PF68 has been proven by many research groups in both mammalian and insect cell lines [1–14, reviewed in 15]."

Pluronic F-68 is a polyoxyethylene-polyoxyproplylene- polyoxyethylene triblock copolymer which is evidenced by Nemeth et al., wherein said polyoxyethylene-polyoxyproplylene- polyoxyethylene triblock copolymer which are noted to be widely used as antifoaming agents (meets the limitations of claim 12) – see p. 152, 1st column, 2nd paragraph, and 2nd column, 4th paragraph.

Therefore it would have been obvious to one of ordinary skill in the art at the time the invention was made to use/add Pluronic F-68 in the cell culture medium in the methods as taught by Moller et al. when said method is being used to cultivate animal or insect cells. While Moller et al. method is applicable to all cell culture supernatants (their primary example is from yeast), it is very well known that when cultivating animal and insect cells that the additive Pluronic F-68 (or similar block nonionic copolymers)

must be added to the cell cultures in order to prevent the cells from essentially dieing in the fermentation process (and hence no production of the protein of interest is possible). For these reason, one skilled in the art would also be motivated to add said Pluronic F-68 to the cell culture medium, when said skilled artisan is cultivating animal or insect cells. There would be a significant expectation of success in performing Moller et al. methods of adjusting the conductivity of *any* cell culture supernatant (e.g. an aqueous starting material, inclusive of animal or insect cells) to less than 6.0 mS/cm, subjecting said supernatant to ultrafiltration followed by diafiltration to adjust the conductivity to less than 2.2 mS/cm (which is "about" 1.5 mS/cm) and then reconcentrate the second retentate by way of another ultrafiltration step because Schulz et al. explain that ultrafiltration and diafiltration are one of the most efficient processes recommended as a first step in downstream procedures for the recovery of proteins from mammalian cell cultivation and thus use of Pluronic F-68, a polyoxyethylene-polyoxyproplylene-polyoxyethylene triblock copolymer as evidenced by Nemeth et al., is essential in this process and because Palomares et al. teach that said Pluronic F-68 is also vital for insect cell cultures in an analogous manner to mammalian cell cultures.

Thus the claims are deemed to be *prima facie* obvious over the teachings of Moller et al. and in view of Schulz et al. and Palomares et al. as evidenced by Nemeth et al.

Response to Arguments

4. Applicant's arguments filed 06 March 2009 have been fully considered but they are not persuasive.

Applicants traverse the rejection for the following reasons. In the first instance, the main argument with the primary teaching of Moeller et al. seems to be that the method is taught using only one protein as an example. Applicants state that:

> In contrast, Moeller et al. discloses a process in which a protein is concentrated by uttrafiltration using membranes in which the stated molecular weight cut-off of the membrane is higher than the molecular weight & the protein to be retained by the membrane. Col. 3, lines 36-43.
>
> Moeller et al. provides ***only one*** example of this.
>
> AND: This is hardly a generalized teaching, but rather is a specific showing of one example going against the conventional teaching that filter pores should be smaller than the molecule they are to retain.
>
> AND: Moeller et al. provide no theory or explanation for why hirudin defies theory under these conditions as is retained.

- See Applicants' REMARKS, pp. 4, paragraph 3 to p. 5, paragraph 3.

However, the examiner is unaware of any sort of legal standard which states that an obviousness teaching is rendered obsolete or inapposite merely because it only utilizes a single example as Applicants are seemingly suggesting. Applicants are correct in stating that Moeller et al. are teaching standards which are going against the conventional standards of what is known in the art, however, it is clearly apparent that this standard is the *same* standard of what is outlined in the instant methods and clearly is thus taught in the prior art.

Applicants subsequently recite what the methods recite and also state Moeller et al. does not teach ultrafiltration with an organic polymer. Because Moeller et al. does not teach an organic polymer, it clearly does not teach that the polymer is a nonionic

block copolymer (claim 6), Pluronic® F-68 (claim 7 in generic terms), or selected from polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymers, polyethylene glycol, and antifoam polymers (claim 12). And that the Examiner admits to as much. (see Remarks, p. 6, 2nd paragraph)

However, this is precisely why the claims have been rejected under 35 U.S.C. 103(a), and not under 35 U.S.C. 102; the Examiner is well aware that Moeller et al. does not teach each and every element of the claimed invention, e.g. addition of F-68 or other nonionic block co-polymers. However, this is why secondary references of Schulz and Palomares et al. (as evidenced by Nemeth et al.) are utilized, e.g. Schulz et al. teach the advantage of ultrafiltration and Palomares et al. teach that F-68 is one of my the most common additives to mammalian and insect cell cultures and is known to be vital and essential for successful cultivation of said cells and that this has been known and is common knowledge in the art and has been so for a very long time. Nemeth et al. evidence that F-68 is a polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymer and that these are commonly used as anti-foaming agents.

Applicants also argue that Moller et al. offer no reason, guidance or explanation of why or how their method works and thus one skilled in the art would expect it only to work for their single example of hirudin in yeast. However, clearly Moller et al. DO state that this is an exemplary example and that:

"***The need to isolate a protein or peptide from fermentation medium typically arises in the context of recombinant microorganisms transformed with suitable expression vectors***. Desired proteins or peptides, for the present ultrafiltration process, are typically a recombinantly produced protein or

peptide. Often, the desired protein or peptide may contain an overall charge due to a greater overall concentration of basic or acid amino acid residues. A preferred protein or peptide contain an overall positive or negative charge of 2 or larger. Also preferred is a protein or peptide with an overall charge of 4 or larger.

The utility of this process may be demonstrated by the isolation of the thrombin inhibitor hirudin, a single-chain protein with 65 amino acids, from the culture supernatant of the yeast strain Saccharomyces cerevisiae modified
by genetic engineering." - see Column 1, lines 19-35.

Thus, they are clearly stating that this is just an example which can be utilized in any other recombinant expression system wherein production of proteins or peptides is desired. It is noted, furthermore, that such systems are well known in the art to include bacterial, mammalian and insect cells. Applicants again state that there would be no reason or motivation to go from yeast to other expression systems. However, the examiner disagrees with this assertion because it is well known and established in the art that successful recombinant production or proteins is often expression system orientated, e.g. insect cells may work better for certain proteins than bacterial or yeast cells, or vice-versa. The Moller et al. method and the instant methods are not limited by the expression system whatsoever, rather the methods are utilized subsequent to an efficient expression system already having been established. In addition, the motivation clearly would come from the desired recombinant protein being produced. One skilled in the art fully understands that the expression system can be vital for the amount of product desired and usually has this figured out prior to arriving at the fermentation and purification process, as noted. When one utilizes yeast expression

systems, for instance, the need to add subsequent F-68 for example, is minimized. However, if the desired protein expression system is best suited to insect OR mammalian cell systems, adding said F-68 is vital essential and obvious gibven the teachings of Shculz, Polamares (and Nemeth et al.).

Furthermore, the examiner also disagrees with Applicants assertions that there is no explanation of why only hirudin would work in the Moller et al. system. There is a sufficient explanation which can be transcended to most any protein and Applicants have merely overlooked said explanation:; "Often, the desired protein or peptide may contain an overall charge due to a greater overall concentration of basic or acid amino acid residues. A preferred protein or peptide contain an overall positive or negative charge of 2 or larger. Also preferred is a protein or peptide with an overall charge of 4 or larger."

In addition:

> "Thus, The present invention accordingly also relates to a process for prepurification of a cell-free culture medium comprising a peptide by means of ultrafiltration on a membrane, wherein the stated molecular weight cut-off of the membrane is approximately two to approximately five times, preferably approximately three to approximately four times, the molecular weight of the peptide or protein to be retained by the membrane. Ultrafiltration membranes are characterized, in addition to other properties, by their nominal molecular weight cut-off. All the molecules larger than the molecular weight cut-off of a particular membrane are generally retained and those smaller than the molecular weight cut-off level generally pass through the membrane. Atkinson and Mativuna; Biochemical Engineering and Biotechnology Handbook, Chapter 17, "Product Recovery

Processes and Unit Operations", 2nd Ed., Stockton Press 1991, NY, USA see page 978. " see col. 3, lines 35-50.

The entire section prior to this state is bound in theory AND explanation of the process and how it does work and will work and the assertion that there is no motivation to go from yeast to mammalian or insect cells (which the examiner notes is only required for claim 9) is not convincing.

Applicants also suggest that one would not be motivated to add Pluronic F-68 to insect or mammalian cells because Schulz et al. teach that is can foul the membrane and cause damage to the downstream processing of pharmaceutical proteins, although it is noted that Palomares et al. do in fact teach that F-68 is an effective shear protective agent.

While it is noted that the additional time may be required for the addition of F-68, the cost-benefit of the addition of said nonionic block copolymer clearly out weighs this additional time. Without the addition of said F-68, cells are sheared and thus destroyed and the loss of product is considerable. As noted by Palomares et al., addition of F-68 to the culture medium *increased* the overall product yield considerably, 10x to 20x as compared to without it (see abstract). This alone is motivation enough. However, it is also noted by Polamers et al. "The results shown here indicated that Pluronic F-68 physically interacts with cells in a direct, strong, and stable mode, not only protecting them from hydrodynamic damage, but also modifying their capacity for recombinant protein and virus production." (See last line of abstract).

Finally, Applicants argue that Claim 2 of the present invention recites in step 2 "adjusting the conductivity of the first retentate solution such that precipitation of the solution components induced by the organic polymer is substantially prevented or substantially reversed ...." and note that the Office Action relies upon the disclosure in Moller et al. of a diafiltration step in the isolation of hirudin as suggesting this step. Office Action, p. 4. Yet Moeller et al. teach diafiltration for desalination. If salinity is not a problem, then this step could be ignored. For example, the skilled person would be motivated to ignore this diafiltration step if salinity is not a problem for his or her process, such as when subsequent chromatography steps can be performed under the salinity conditions of the retenate. To establish that the invention would have been obvious, the Patent Office must show that not only would the skilled person be motivated to modify Moeller et al. to an insect or mammalian cell expression system and use an organic polymer such as Pluronic® F-68, but also that the skilled person would then be motivated to continue with a diafiltration step that was only taught for desalination. Such a showing has not been made. (see Remarks, p. 7, last paragraph).

It is asserted that the Office has efficiently established that modifying the expression system could easily be made, while also noting that Applicants reliance on this argument really only pertains to dependent claim 9. Nonetheless, as asserted modifying the exemplary method as noted by Moller et al. to other systems is clearly established in the art by desired expression system which is not limited by the taught methods. In addition, Applicants are asserting that the decrease in salinity, e.g.

desalination can be ignored, ***IF*** salinity is not a problem. They are merely inserting subsequent method steps into the claim which are not there and which are clearly not taught by Moller et al. (rather, Moller et al. DO teach the desalination/reduction in conductivity of the retentate as is claimed). Should Applicants wish to argue that such prophetic downstream processing steps as being essential or included/excluded from the instant claims, then it is suggested that such limitations somehow be included in the instant method steps.

With regards to claims 10 and 11, it is the result of the extra ultrafiltration step that can be utilized which results in a higher concentration of molecules (col. 5, line 7-48). It is noted that the concentration limits state for the concentrated molecule are well within the noted standards and capabilities of the ultrafiltration method in and of itself and these concentration levels are well understood in the art (see for examples, US 4900673, Ex. 4, US 5108920, Fig. 7 and US 51567602, Ex. 7.1).

Conclusion

5. No claims are allowed.

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUZANNE M. NOAKES whose telephone number is (571)272-2924. The examiner can normally be reached on 7.00 AM-3.30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SUZANNE M. NOAKES/
Primary Examiner, Art Unit 1656
01 June 2009